do Nascimento, C. P., Maretto, G. X., Marques, G. L. M., Passamani, L. M., Abdala, A. P., Schenberg, L. C., Beijamini, V., & Sampaio, K. N. (2017). Methamidophos, an Organophosphorus Insecticide, Induces Pro-aggressive Behaviour in Mice. *Neurotoxicity Research*, *32*(3), 398-408. https://doi.org/10.1007/s12640-017-9750-9

METHAMIDOPHOS, AN ORGANOPHOSPHORUS INSECTICIDE, INDUCES PRO-AGGRESSIVE BEHAVIOUR IN MICE

Cristina Paula do Nascimento[a], Gabriella Xavier Maretto[b], Graziany Leite Moreira Marques[a], Luciana Mesquita Passamani[a], Ana Paula Abdala[c]; Luiz Carlos Schenberg[b], Vanessa Beijamini[a], Karla Nívea Sampaio[a]

[a]Department of Pharmaceutical Sciences and [b]Department of Physiological Sciences, UFES, Av. Marechal Campos, 1468, Maruípe, Vitória, ES, Brazil
[c]School of Physiology and Pharmacology, Medical Sciences Building, University of Bristol, Bristol BS8 1TD, UK

Running Title: Methamidophos induces pro-aggressive behaviour in mice

Vanessa Beijamini and Karla Nívea Sampaio share equal contribution to this report

Correspondence to:
Dr. Karla Nívea Sampaio
Federal University of Espírito Santo
Department of Pharmaceutical Sciences
Health Sciences Center
Av. Marechal Campos, 1468, Maruípe, Vitória, ES, Brazil
ZIP: 29043-900
e-mail: kniveas@yahoo.co.uk; karla.sampaio@ufes.br
Tel: 55 27 3335 7460
Fax: 55 27 3335 7345

Acknowledgments

This work was supported by a grant from FAPES (n° 3112011/2005). FAPES is a public research foundation and had no involvement with the study design, collection, analysis and interpretation of data. We acknowledge the Multiuser Laboratory of Biomolecular Analysis UFES (LABIOM) in the Health Sciences Center of the Federal University of Espírito Santo, Brazil, for providing the equipment necessary for the brain AChE assay.

Abstract

Although evidence indicates that exposure to organophosphorus (OP) pesticides induces neurobehavioral disorders, little is known about the effects of OP on aggressive behaviour. Our study investigated the effects of repeated exposure to an OP pesticide, methamidophos, on the isolation-induced aggressive behaviour in mice. Forty seven male mice were individually housed for a month. Socially isolated animals were then confronted with a standard non-isolated opponent for 15 minutes (pre-treatment trial), and the latency and frequency of aggressive and general exploratory behaviours were recorded. Based on the presence of attack behaviour in the pre-treatment trial, mice were classified as isolation-induced aggressive and non-aggressive. All mice were then treated for seven days with methamidophos (3.5 mg/kg/day, n=22, ip) or saline (1 mL/kg/day, control group, n=25, ip) and a second trial performed. Repeated exposure to methamidophos induced attack behaviour in non-aggressive mice. The treatment with methamidophos also decreased plasma butyrylcholinesterase and brain acetylcholinesterase activity. These results suggest that methamidophos has a pro-aggressive effect on socially isolated mice.

Keywords: methamidophos, aggressive behaviour, mice, butyrylcholinesterase, acetylcholinesterase

1. Introduction

Methamidophos (O,S-dimethyl phosphoroamidothioate) is a highly toxic organophosphorus (OP) pesticide widely used worldwide (Caldas et al. 2011; Caldas et al., 2006; Gray, 1982; Lu 2010; Robinson and Beiergrohslein, 1982; Yu et al. 2015). Among the possible sources of human exposure, the diet route poses a substantial risk and recent reports show that methamidophos is the highest-ranking pesticide exceeding by 100% the acceptable daily intake (Melnyk et al. 2016). Occupational exposure is also widespread, particularly amongst farmers, where the combination of absence of personal protection equipment during handling and a high frequency of usage may lead to acute and long term poisoning (Recena et al. 2006b; Recena et al. 2006a). Worldwide around 350,000 cases of self-poisoning with pesticides are recorded per year, and it is estimated that nearly 30% of global suicides are due to pesticide self-poisoning, with most cases recorded in low and middle income countries (Gunnell et al., 2007; Hulse et al., 2014).

Acute exposure to this OP induces severe systemic disturbances, mostly associated with acetylcholine (ACh) accumulation on synaptic clefts due to inhibition of acetylcholinesterase (AChE) activity (Chowdhary et al. 2014; Ecobichon, 2000; Jeyaratnam, 1990). The symptoms observed after acute poisoning mainly result from overstimulation of cholinergic receptors present in synapses of the autonomic nervous system, central nervous system and neuromuscular junctions (O'Malley 1997; Eddleston et al. 2008b). Although acute symptoms are the most alarming because of their severity and even fatality, prolonged exposure to non-lethal doses of OP also presents with negative neurological effects [see Mackenzie Ross et al., 2013 for review].

Central nervous system exposure to OP is high due to their lipophilicity which confers a great ability to cross the blood brain barrier (Ferrer 2003). Measurements of brain AChE activity combined with anatomical and behavioural studies provide evidence of OP-induced neurological effects in animal models (Ali et al. 1980; Socko et al. 1999; Sánchez-Amate et al. 2001a; Pelegrino et al. 2006; López-Crespo et al. 2007a; Lima et al. 2009a). Pelegrino and colleagues (2006) showed that repeated administration of sublethal doses of methamidophos induces atrophy of the molecular layer of the parietal cortex of rats. Neurobehavioural changes such as reduction in locomotor and exploratory activity (Ali et al. 1980; Socko et al. 1999; López-Crespo et al. 2007b), as well as depressive (Lima et al. 2009b) and anxiety-like behaviour (Sánchez-Amate et al. 2001b) have also been described after experimental exposure to OP pesticides. Although a number of studies point to important effects on behaviour, the effects of these compounds on aggressive behaviour remains poorly explored.

Clinical reports also provide evidence for OP-induced neurological disorders. Humans exposed to OP acute toxicity present a higher risk of depression, suicide or cognitive impairment (Savage et al. 1988; Rosenstock et al. 1991; Stallones and Beseler 2002; Beseler et al. 2006). A higher percentage of anxiety and depression was observed in sheep farmers chronically exposed to OP pesticides when compared with non-exposed individuals (Stephens et al. 1995; Mackenzie Ross et al. 2010). Likewise, an increased risk of depression (Beseler et al. 2008) and suicide (Parrón et al. 1996; Wesseling et al. 2010) has also been described among individuals chronically exposed to OP when compared with subjects with similar socioeconomic and demographic characteristics.

A large number of the aforementioned psychiatric disorders show association with aggressive behaviour (Haller and Kruk 2006), and there are some reports of unprovoked aggressive behaviour, including two homicides, following short and long term exposure to cholinesterase inhibiting pesticides (Devinsky et al. 1992). Further, experimental studies show that rats chronically exposed to the OP dichlorvos exhibited marked aggression compared with control animals (Sarin and Gill 1998). Despite the evidence mentioned above, the effects of short term exposure to OPs on aggressive behaviour remain poorly understood. This has implications for the prevention of early psychiatric episodes, particularly among populations frequently exposed to OPs. Better understanding of this feature of OP exposure is highly relevant for socio-economic policy making in impoverished countries whose economies are dependent on agriculture. We hypothesized that short term repeated exposure to non-lethal doses of OP would promote aggressive behaviour in mice. In the present work, we tested the effects of subacute exposure to sublethal doses of methamidophos in a murine model of isolation-induced aggression (Malick 1979; Miczek 1983; Miczek et al. 2001; Haller and Kruk 2006). We also monitored locomotor activity, brain acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) activity.

2. Material and Methods

2.1. Animals

Experiments were performed in male Swiss mice weighing between 25 and 35 g bred in-house. All experiments were performed in accordance with the Biomedical Research Guidelines for Animal Welfare, as stated by the Federation of the Brazilian Society of Experimental Biology and the project was approved by the Ethics Commitee in Animal Experimentation (CEUA-UFES n° 058/2010). Mice were housed in cages in a temperature and humidity controlled room with a 12 h light/dark cycle. Standard mice chow and tap water were available *ad libitum*.

2.2. Dose and Treatment

Methamidophos (*O,S*-dimethyl phosphoramidothioate; 60 percent m/v, Tamaron, Bayer) was diluted in saline (0.9 %) for administration by means of intraperitoneal injections. The i.p route was chosen to allow accurate and efficient delivery of the chosen dose and to reduce exposure variability. A lethality curve to methamidophos was previously determined in mice (Maretto et al. 2012) and a sublethal dose was chosen for the treatment protocol. Mice were treated with repeated administration of methamidophos (MTP, 3.5 mg/kg/daily, i.p., n=22) for seven days and a similar regimen was adopted for a control group treated with saline (SAL, 0.9 %, 1 mL/kg/day, i.p., n=25). The seven day treatment period was based on studies from Amr *et al.*(1997) that investigated psychiatric disorders in a population of pesticides formulators exposed to OPs on a daily basis for at least 9 months per year in the last two years. Considering that the ratio between human and mouse lifespan is approximately 40:1 (Dutta and Sengupta 2016), we adopted a 7 day treatment period to produce an equivalent exposure to 9 months in humans. Assignment to methamidophos or saline was randomized, and the experimenter was blinded during treatment, data collection and analysis.

2.3. Social isolation-induced aggression

Long-term social isolation in mice induces many behavioural changes that resemble those seen in depressive and anxiety disorders (Malick 1979; Fone and Porkess 2008). It also induces territorial and aggressive behaviour, resulting in attacks against an opponent (Malick 1979). Socially isolated mice were individually housed for 28 days in cages measuring 30 x 20 x 13 cm. The standard opponent mice were housed in groups of five in cages measuring 41 x 34 x 16 cm. After the isolation period, each isolated mouse was individually placed in a neutral cage (41 x 34 x 16 cm) together with a weight-matched non-isolated mouse (pre-treatment trial), i.e. a standard opponent. The latency and frequency of attacks, tail rattling and general exploratory behaviours (number of rearings and self grooming) were recorded over a 15 min period (Brain and Poole 1974; Brain 1980). Animals were classified as aggressive or non-aggressive based on the presence or absence of an attack in this first trial. A second trial (i.e. post-treatment trial) was conducted 24 hours after the last injection of methamidophos or saline, i.e. 8 days later. The same standard opponent was used in the pre-treatment and post-treatment trials for each isolated animal tested.

2.4. Open field test

In order to evaluate whether methamidophos treatment could induce exploratory behaviour dysfunction that might interfere with the isolation-induced aggression test, we performed the open field test in a separate group of animals treated with saline or methamidophos under the same dose regimen (n=10/group). The open field apparatus consisted of a circular arena with a peripheral and a central area (total area= 1256 m², radius of the external circle = 20 cm, radius of the internal circle = 11.8 cm) divided in 12 spaces by a grid cross on the floor (4 central and 8 peripheral), each space corresponding to a 105 cm² dimension. Twenty-four hours after the last injection of saline or methamidophos, each mouse was positioned in the centre of the apparatus and monitored for a period of 10 minutes. The total number of peripheral and central squares crossed was recorded. In addition to providing a measurement of general locomotor activity, which could interfere with other behavioural measures, preference or avoidance of central squares may also provide an evaluation of the anxiety level (Prut and Belzung 2003).

2.5. Sample collection

After the open field test, animals were decapitated, and brain and blood were harvested. Blood samples were collected in heparinized plastic microtubes and centrifuged at 1792 G for 10 minutes at 4°C (SL-5AM® - Spinlab Scientific, South Korea) to obtain plasma. Plasma samples were stored at -20°C until dosage assays were perfomed. Brain tissue was quickly removed and stored in centrifuge tubes at -80°C until the day of the assay.

2.6. Plasma butyrylcholinesterase (BChE) activity

Plasma activity of BChE or AChE is frequently used for the diagnosis of OP exposure (Eddleston et al. 2002). BChE activity is more easily measured and is used in the clinical setting as a useful tool for

indicating likelihood of exposure and for monitoring OP elimination (Eddleston et al, 2008). We aimed to verify that the sublethal dose chosen exceeded the occupational exposure level as stated in the Brazilian National Regulatory Law (Brasil 1978) for occupational exposure to cholinesterase inhibitors. According to this law, cholinesterase inhibitors may produce a maximum of 50% inhibition of the BChE. Therefore, the BChE assay was adopted as a screening tool to verify the effectiveness of the dose chosen in exceeding the maximum allowed exposure level. Plasma BChE activity was measured using a commercial kit (Doles, Brazil), which uses propionylthiocholine as substrate, and follows a colorimetric method described by Ellman et al. (1961) and modified by Dietz et al. (1973). BChE activity was measured in International Units (I.U./mL) and expressed as percentage of control activity. One I.U. of cholinesterase is the amount of enzyme that hydrolyses one µmol of substrate/minute/mL of serum at 37°C.

2.7. Brain acetylcholinesterase (AChE) activity

Whole brain AChE activity was determined using Ellman's *et al.* (1961) method, modified by Lassiter *et al.* (2003) and Pires *et al.* (2005). Briefly, whole brains were weighed, homogenized in phosphate buffer with Triton-X 1% (proportion of 1 ml of phosphate buffer to 20 mg of tissue) and centrifuged at 7800 G for 5 minutes at 4°C. A volume of 135µl of supernatant was transferred to a cuvette containing the following reagents: 35µl of 5mM dithio-bisnitrobenzoic acid (DTNB), 10µl of 75mM acetylthiocholine (ATCh) and 820µl of 0.1M phosphate buffer (pH 8.0). The colour development was recorded at 412 nm, using a spectrophotometer (Evolution 300 PC, Thermo Scientific, USA). Protein concentration in brain homogenates was quantified using a Bradford assay. AChE activity was calculated in µmoles of ATCh hydrolyzed per hour per mg of protein. AChE activity was expressed as percentage of control activity and measured values in µmol/h/mg of protein.

2.8. Statistical Analysis

Results are reported as mean ± standard error of the mean (S.E.M.). Data from the social isolation-induced aggression test were analysed using a two-way ANOVA for repeated measures followed by Bonferroni's post test, with methamidophos or saline treatment as the between-subject factor, and pre- and post-treatment trials as the within-subject factor. Data from the open field test, brain AChE and plasma BChE activity were analysed using paired Student *t*-test. $P<0.05$ was considered to be significant. All analyses were performed using Graphpad prism software (Graphpad prism 5.0, USA).

3. Results

3.1. Social isolation-induced aggression

In the pre-treatment trial, performed after the isolation period, it was observed that some mice became aggressive, which could be identified by the presence of attacks against the opponent mouse, and some mice did not. Based on this observation, mice were grouped as isolation-induced aggressive and non-aggressive, and mice within each group were randomly assigned to either methamidophos or saline. According to this classification, we compared data from: (i) all isolated mice grouped together,

(n=47); or (ii) as separate groups of pre-treatment aggressive (n=25) and non-aggressive (n=22) mice. Figure 1 shows summary data from the grouped mice. No statistical difference was observed for all parameters analysed either for the repeated measures or for the treatment, and there was no interaction between treatment and time (for statistical values see Table 1). When analysing aggressive mice separately, we found no statistical difference between saline and methamidophos treatment (Figure 2, Table 1). However, non-aggressive mice showed significantly higher number of attacks over time [$F_{(40,1)}$=5.287; p=0.0268], compared to saline controls [$F_{(40,1)}$=4.328; p=0.0439], and there was a significant interaction between factors [$F_{(40,1)}$=4.328; p=0.0439] (Figure 3). Non-aggressive mice treated with methamidophos did not differ in number of attacks from aggressive mice in the pre-treatment trial (p>0.05). All other parameters evaluated in the non-aggressive group after methamidophos treatment were not statistically different (Table 1).

3.2. Open field test

The results of the open field test are presented in Table 2. No statistical difference was observed between the different treatment groups for peripheral squares crossed (t_{18}=-0.936, p=0.362). However, there was a tendency to reduction in the number of central squares crossed in the methamidophos-treated group when compared to saline-treated animals (t_{18}=2.08, p=0.052).

3.3. Brain AChE and Plasma BChE activity

Repeated administration of a sublethal dose of methamidophos induced a significant reduction of 72% on BChE activity when compared to saline group (t_{17}=6.97, p<0.001; Figure 4A). BChE activity was 10.73 I.U./mL in the saline, and 3.07 I.U./mL in the methamidophos group. Methamidophos treatment also significantly decreased brain AChE activity when compared to saline group (t_{18}=3.00, p<0.01; Figure 4B). Mice treated with saline exhibited AChE activity of 4.04 µmol/h/mg of protein and those treated with methamidophos exhibited AChE activity of 3.16 µmol/h/mg of protein.

4. Discussion

In the present study, repeated administration of methamidophos induced a pro-aggressive behaviour in mice that did not present isolation-induced aggressive behaviour before OP treatment. This effect was observed in absence of any interference in the peripheral locomotor activity evaluated through the open field test. Mice treated with methamidophos also showed a trend towards reduction in locomotor activity in the central squares, suggesting that exposure to methamidophos had an anxiogenic-like effect. It is noteworthy that, although a sublethal dose of methamidophos was employed, final plasma BChE activity resembled that of acute OP poisoning. BChE is a sensitive marker of exposure to OPs (Eddleston et al. 2008a). Plasma levels of BChE achieved the diagnosis criteria for cholinesterase inhibitors exposure stated by the Brazilian National Regulatory Law (Brasil 1978), which sets 50% inhibition of BChE activity as a maximum biological exposure level. Although, BChE inhibition does not straight correlate with clinical features in OP-poisoned patients, the independent measurement of brain AChE confirms that methamidophos treatment significantly

decreased whole brain AChE activity in this study. In fact, Singh (1985) has demonstrated that brain samples are more sensitive to methamidophos than plasma samples.

It is important to consider, however, some methodological limitations of the dosage regimen employed in the present study. . The measurement of brain AChE activity by the Ellman's method without usage of a specific inhibitor of BChE does not allow us to exclude BChE activity in our brain samples. Notwithstanding, according to Vellom et al. (1993), AChE promotes rapid catalysis of the substrate used, acetylthiocholine, while BChE shows far less selectivity for the size of the acyl group. This is clearly demonstrated by the kinetic constants (Kcat/Km ratio) for the catalysis of acetylthiocholine by AChE (4.4×10^9) and by BChE (0.24×10^9). Additionally, Lassiter and colleagues (2003) showed that only 12% of overall cholinesterase activity was reduced in rodent brain samples after inhibition of BChE with iso-OMPA. This indicates that almost 90% of cholinesterase activity measured in rodent brains relates to AChE.

Methamidophos, like other OP pesticides, is a cholinesterase inhibitor. Evidence suggests that cholinesterase inhibitors may increase aggression in animals and humans. Studies performed by Allon *et al.* (2005) reported that rats acutely exposed to sarin vapour, a potent AChE inhibitor, exhibited signs of aggression and weight loss. Although Allon *et al.* (2005) did not use a specific test for evaluation of aggressive behaviour. Mice treated with another OP compound, chlorpyrifos, during gestational and postnatal phases, showed enhanced agonistic behaviour (Ricceri et al. 2006). In rats, 10 days exposure to diisopropylfluorophosphate, an OP pesticide, increased shock-induced aggression (Ray et al. 1989). Moreover, chronic exposure to the OP dichlorvos increased aggressive behaviour in rats while reducing brain AChE activity (Sarin and Gill 1998). Further, physiostigmine, a reversible AChE inhibitor, has been reported to enhance intermale mouse aggression at low doses (Charpentier 1969). In fact, increase in aggression in mice and rats has been previously attributed to cholinesterase inhibitors and muscarinic receptor agonists (Bell et al. 1985). Moreover, there are some case reports of patients exhibiting aggressive behaviour while using donepezil, a reversible cholinesterase inhibitor used for the treatment of dementia (Bouman and Pinner 1998; Bianchetti et al. 2003). Although, the above mentioned reversible cholinesterase inhibitors differ from OP compounds in their kinetics and dynamic properties, there is a common feature between them which is the consequent ACh accumulation in synapses and interference with cholinergic transmission. Devinsky *et al.* (1992) reported episodes of unprovoked aggressive behaviour, including two homicides after OP exposure. Among the cases reported, one occurred after one month of exposure while the other two cases involved more than 3 years of OP exposure. Amr *et al.* (1997) investigated psychiatric disorders in a population of pesticide formulators exposed daily for at least 9 months of the year, and found that irritability was amongst the most frequent symptoms observed. Although no previous studies have investigated the effects of short term repeated OP exposure in the isolation-induced aggression test, the above mentioned evidence are in accordance with our results, in which a pro-aggressive behaviour induced by repeated methamidophos exposure could be observed.

Given the evidence for cholinergic involvement in aggressive behaviour, it is possible that the pro-aggressive behaviour induced by methamidophos treatment can be attributed to cholinergic hyper stimulation in the central nervous system (CNS). This hypothesis is supported by the finding that

Flinders Sensitive Line hypercholinergic rats are significantly more aggressive (Pucilowski et al. 1990). Moreover, cholinomimetic drugs administered into cerebral ventricles of cats elicited affective type of aggression (Beleslin and Samardzić 1979). Likewise, injections of acetylcholine into the hypothalamus and periaqueductal grey matter, brain areas involved in mediating aggressive behaviour, led to defensive rage in cats (Allikmets 1974). Additionally, carbachol injection into the lateral hypothalamus elicited killing behaviour in rats (Smith et al. 1970), while scopolamine, a muscarinic antagonist, reduced aggressive behaviour in mice (Winslow and Camacho 1995). Although we did not measure AChE activity in specific brain structures, the reduction in whole brain AChE activity after methamidophos treatment could generate cholinergic hyper stimulation in brain areas mediating aggressive behaviour. Nevertheless the central cholinergic hyper stimulation seems to be an important mechanism involved in the effects observed after the methamidophos treatment, recent finds on the ghrelin peptide and plasma BChE activity points alternative mechanisms for the aggression effects mediated by the OP exposure (Chen et al. 2015). Knockout mice with plasma BChE gene deletion exhibited increased levels of ghrelin as well as increased levels of aggression (Chen et al. 2015). In fact, previous *in vitro* studies had shown that purified plasma BChE promoted hydrolysis of the ghrelin peptide (De Vriese et al. 2004). Interestingly, overexpression of BChE led to low ghrelin levels in the blood stream and reduced aggression and social stress in mice (Brimijoin et al. 2015). Although caution should be exercised when drawing comparisons between our study and genetically modified mice, it is possible that the large inhibition of plasma BChE activity induced by methamidophos treatment could increase ghrelin levels, which could account for the increase in aggressive behaviour in the present study. Indeed, a recent study using chronic administration of another OP, chlorpiryfos, showed an increase in plasma levels of ghrelin in mice (Peris-Sampedro et al. 2015).

Methamidophos exposure only increased aggression in non-aggressive socially isolated mice. This is in line with previous studies showing that drug-induced changes in aggressive behaviour can be different in mice depending on the aggression level exhibited in the pre-screening test (Felip et al. 2001; Miczek et al. 2002; Lumley et al. 2004; Redolat et al. 2005). Additionally, the anxiogenic-like effect seems to be more obviously detectable in animals with low levels of emotional reactivity (Lisboa et al. 2010). It is possible that this selective effect induced by methamidophos exposure might be associated with a reduction in GABA levels in certain brain areas, which could differently affect sociable and unsociable mice. Sustková-Fišerová et al. (2009) showed that socially isolated non-aggressive mice had almost three times higher levels of GABA in the pre-frontal cortex when compared with aggressive mice. Additionally, chronic exposure to methamidophos was shown to decrease GABA release in cerebral cortex and hippocampal slices (Noriega-Ortega et al. 2011).

Exposure to some OP pesticides, at low doses, also interferes with monoaminergic neurotransmission, particularly via serotonin modulation (Ali et al. 1980; Aldridge et al. 2005; Slotkin et al. 2006). Accordingly, Lima et al. (2011) reported that exposure to low doses of methamidophos affects synaptic transmission by reducing serotoninergic biomarkers in regions such as cerebral cortex and midbrain. Vergnes et al. (1986) also showed that depletion of 5-HT increases aggression in a variety of species in several different social situations. In fact, decreased 5-HT levels in the pre-frontal cortical area are detectable when a resident rat is attacking an intruder (Van Erp and Miczek, 2000). In

the same way, pharmacological manipulation using agonists or reuptake inhibitors aiming to increase 5-HT neurotransmission reduced aggressive behaviour in isolated male mice (Olivier et al. 1989; Sánchez et al. 1993; Sánchez and Hyttel 1994). However, it was beyond the scope of the present study to identify the neurotransmitter systems involved in increased aggression by OP exposure, and further studies will be required to reveal the mechanism.

Our data showed that approximately 50 % of the socially isolated mice did not exhibit aggressive behaviour, i.e. absence of at least one attack episode. Previous studies also described similar proportion of non-aggressive mice in singly housed Swiss mice (Krsiak 1975; D'Amato and Castellano 1989). It is noteworthy, however, that aggressive and non-aggressive mice were confronted with the same standard opponent in the trials tested, which could have interacted with the processing of novelty by the isolated mice. Indeed, conflicting results have been shown after repeated exposure to the same opponent either in the social interaction or in the resident intruder test. Parmigiani and Brain (1983) observed that familiar intruders were less attacked by the resident mouse in the second trial. However, other studies (Brain and Poole 1974; Winslow and Camacho 1995; Koike et al. 2009) observed that repeated exposure to the same intruder raises the aggressiveness of the resident against it. In these studies, the inter-trial time is within minutes to hours after the first trial. In our study, the re-exposure only occurred 8 days after the first trial, which could account for the differences observed. Additionally, (Krsiak 1975) suggest that isolation-induced timidity is stable in repeated interactions. Recently, Hsieh and colleagues (2017) conducted a study in which they showed that the natural social interaction in CD1 mice does not only happen due to novelty. They also showed that testing the mice in one single compartment, similarly to our study, favours aggressive instead of sociopositive behaviours. Finally, if re-exposure alone led to increase in aggressive behaviour in the present study, the same effect would have been observed in the saline control group.

5. Conclusions

Our results show for the first time that non-aggressive socially-isolated mice became aggressive after exposure to sublethal doses of methamidophos. Therefore, alongside many other behavioural changes associated with OP exposure, OP compounds also induce aggressive behaviour that seems to be associated, at least partially, with changes in brain AChE and plasma BChE activity.

Perspectives

While developing countries are the largest buyers of pesticides worldwide, they often do not have effective regulatory policies and enforcement procedures on occupational exposure and environmental contamination. The literature suggests that, amongst other factors, higher suicide rates in rural regions seem to be associated with higher levels of impulsive behaviour and ready access to highly toxic pesticides (Jiang et al. 2013). The evidence from our study, that short term exposure to the OP methamidophos produces a pro-aggressive behaviour, is corroborated by clinical reports on aggressive behaviour related to pesticides exposure (Devinsky et al. 1992; Amr et al. 1997). Our study highlights the importance of potentially implementing psychiatric screening tools such as the General Health Questionnaire (GHQ-28) or the Buss-Perry Aggression Questionnaire (BPAD) (Goldberg et al.

1997; Zivari-Rahman et al. 2012; Alcorn, Joseph et al. 2013) in the populations occupationally exposed to OPs, particularly agricultural workers. This should be done alongside physical exams and measurements of cholinesterase activity. Psychiatric evaluation might be a useful tool to improve early symptom detection and improve vigilance in this working population.

Conflict of interest

The authors declare that they have no competing financial interests, including grant support, employments, patents, payments for expert witness or testimony, personal financial interests and forms of compensation.

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Figures Captions

Figure 1. Grouped data of aggressive and non-aggressive isolated mice in the isolation-induced aggression test, before and after repeated administration of methamidophos (3.5 mg/kg/day, ip, n=22) or saline (Control, n=25). Two-way ANOVA for repeated measures. Data represent mean ± SEM.

Figure 2. Data presented by aggressive animals in the isolation-induced aggression test, before and after repeated administration of methamidophos (3.5 mg/kg/day, ip, n=12) or saline (Control, n=13). Repeated treatment with methamidophos did not change aggressive behaviour in mice that previously exhibited aggressive behaviour induced by social isolation. Two-way ANOVA for repeated measures. Data represent mean ± SEM.

Figure 3. Data presented by non-aggressive animals in the isolation-induced aggression test, before and after repeated administration of methamidophos (3.5 mg/kg/day, ip, n=10) or saline (Control n=12). Repeated treatment with methamidophos increased aggressive behaviour in mice that previously did not exhibited aggressive behaviour induced by social isolation. *$p<0.05$ indicates statistical difference from the methamidophos compared with the control group (Two-way ANOVA for repeated measures followed by Bonferroni's post test). Data represent mean ± SEM.

Figure 4. Plasma butyrylcholinesterase (BChE, panel A) or brain acetylcholinesterase (AChE, panel B) activity of mice after repeated administration of methamidophos (hatched bar, n=10 for BChE and AChE) or saline (control, open bar, n=9 for BChE; n=10 for AChE). BChE and AChE activity are expressed as percentage of control. Comparisons with Student *t* test. **$p<0.001$ and *$p<0.01$ indicates statistical difference compared with the control group.

Table Caption

Table 1. Statistical analysis (Two-way ANOVA for repeated measures) of grouped data, aggressive animals and non-aggressive animals.

Table 2. Parameters evaluated in the open field test in mice treated with repeated administration of methamidophos (3.5 mg/kg/day, ip, n=10) or saline (Control, n=10).

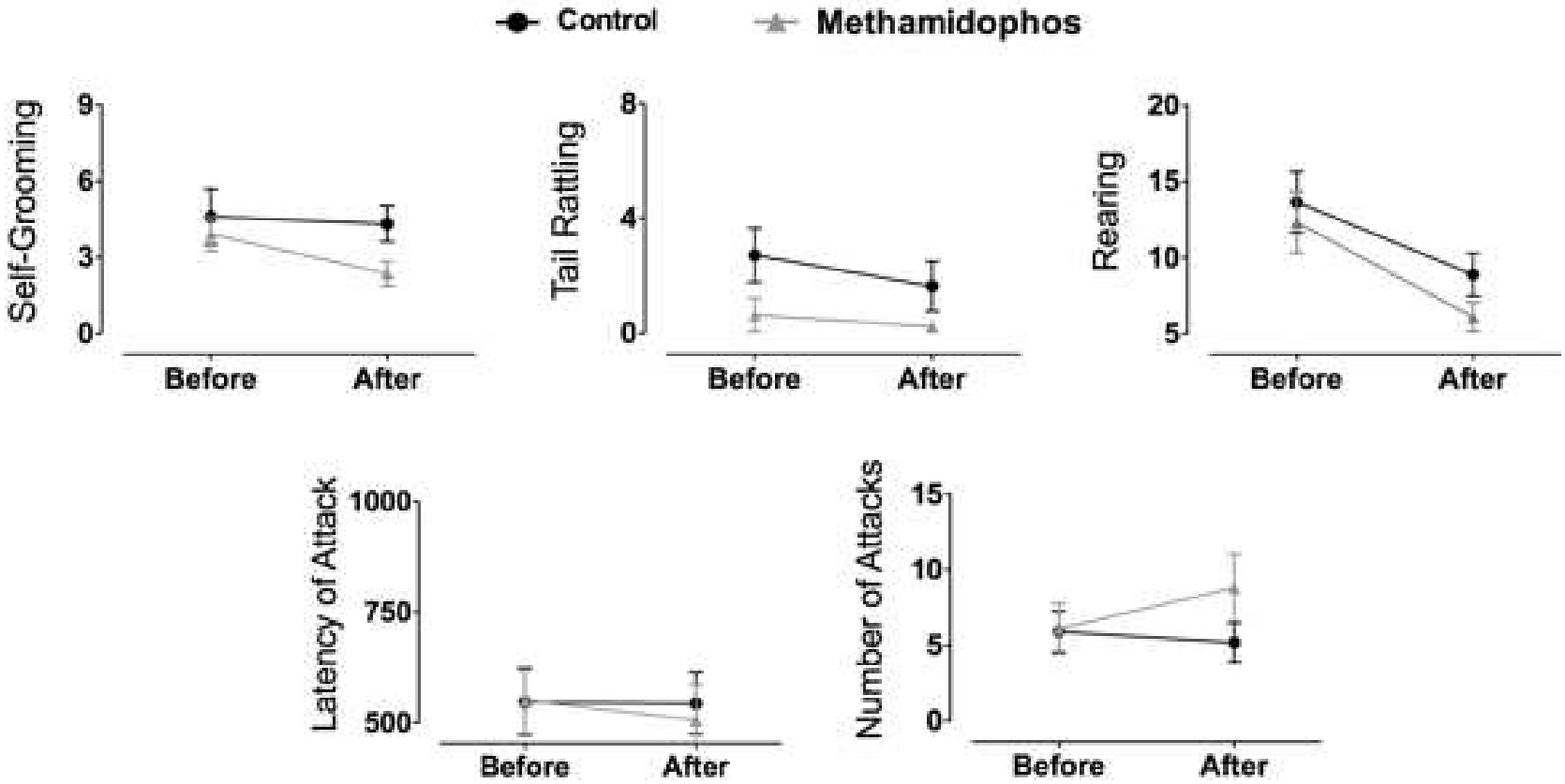
Control
Methamidophos
Self-Grooming
9
6
3
0
Before
After
Tail Rattling
8
4
0
Before
After
Rearing
20
15
10
5
Before
After
Latency of Attack
1000
750
500
Before
After
Number of Attacks
15
10
5
0
Before
After

Figure 1

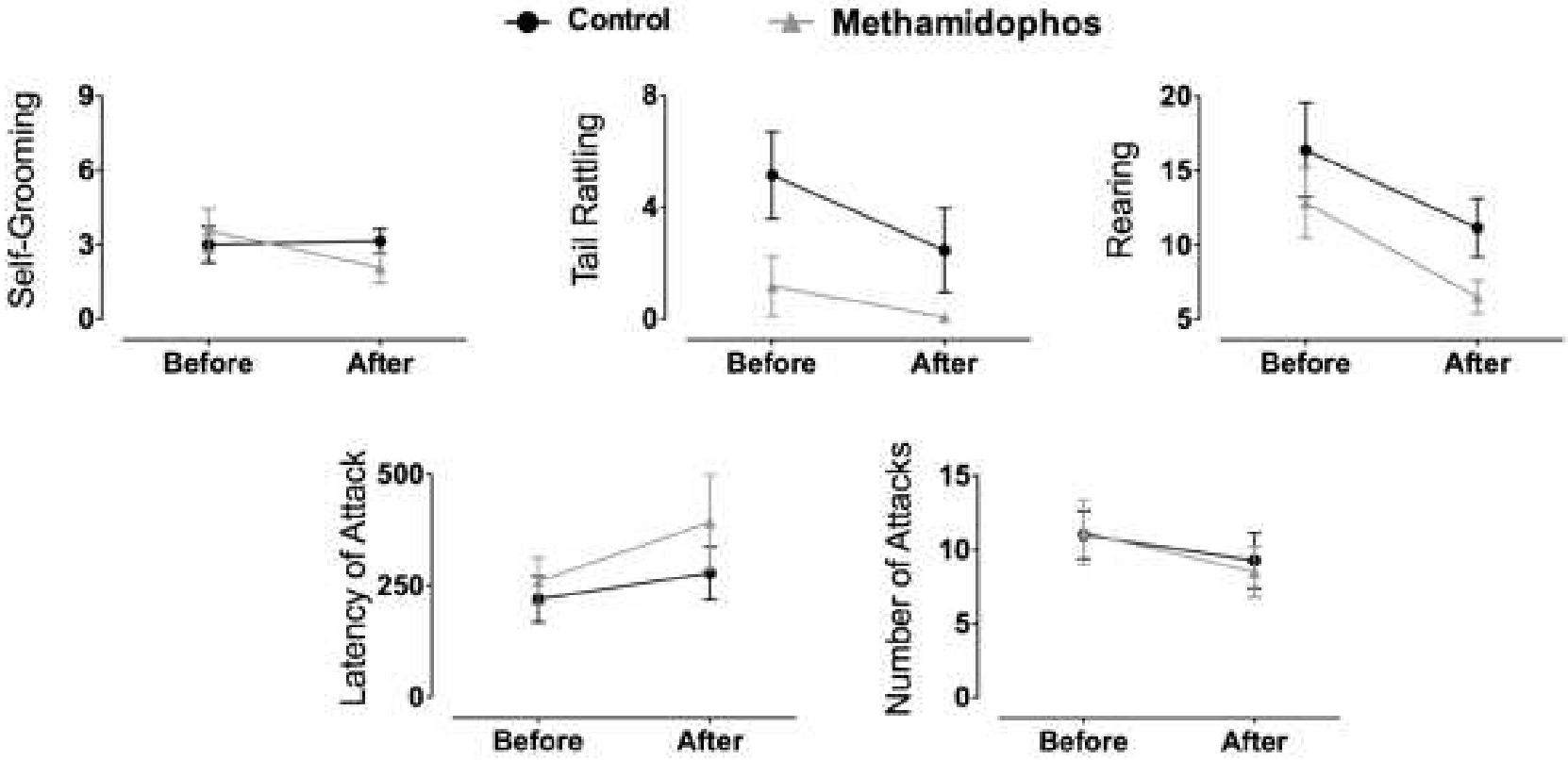
Control
Methamidophos
Self-Grooming
9
6
3
0
Before
After
Tail Rattling
8
4
0
Before
After
Rearing
20
15
10
5
Before
After
Latency of Attack
500
250
0
Before
After
Number of Attacks
15
10
5
0
Before
After

Figure 2

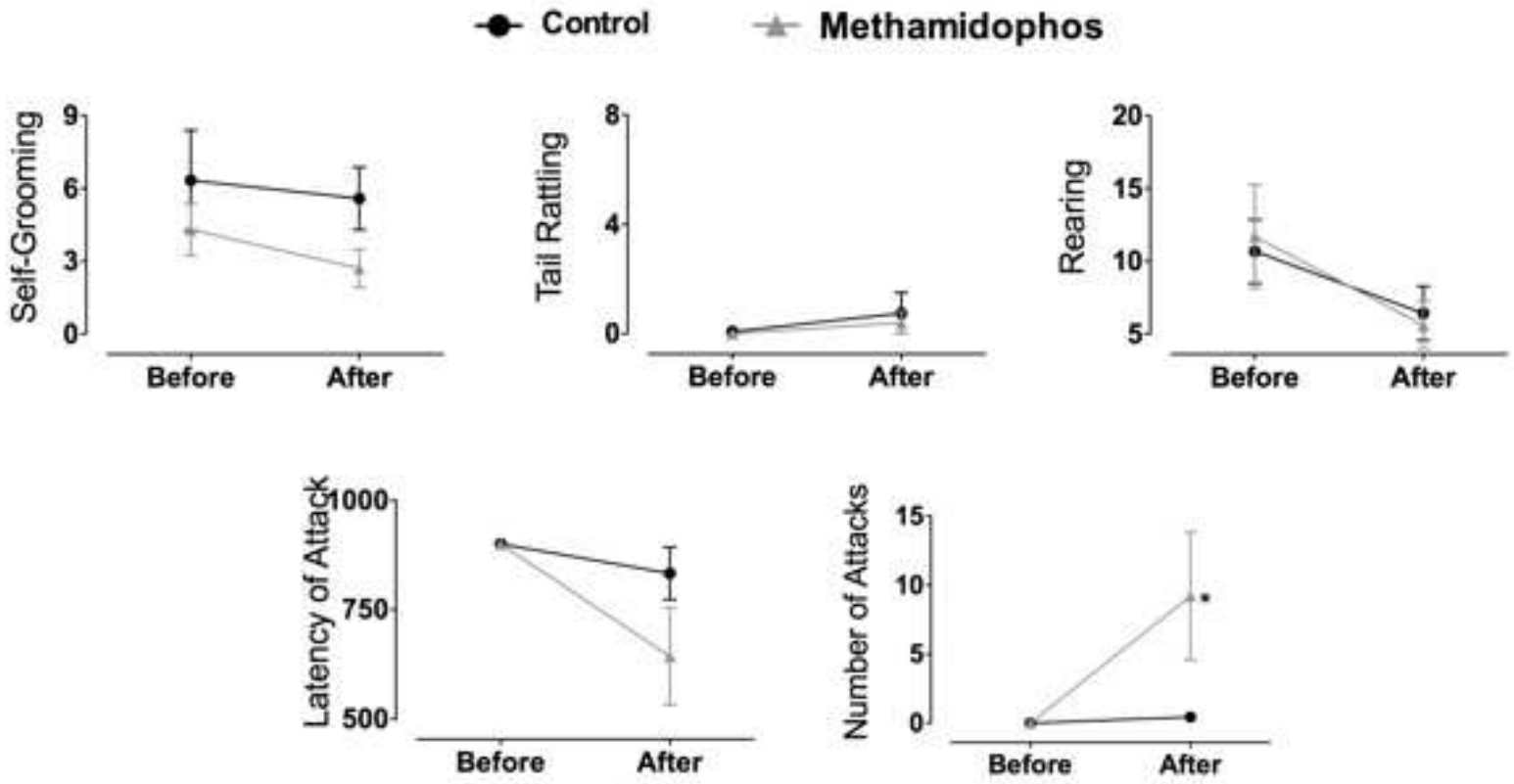
Control
Methamidophos
Self-Grooming
9
6
3
0
Before
After
Tail Rattling
8
4
0
Before
After
Rearing
20
15
10
5
Before
After
Latency of Attack
1000
750
500
Before
After
Number of Attacks
15
10
5
0
*
Before
After

Figure 3

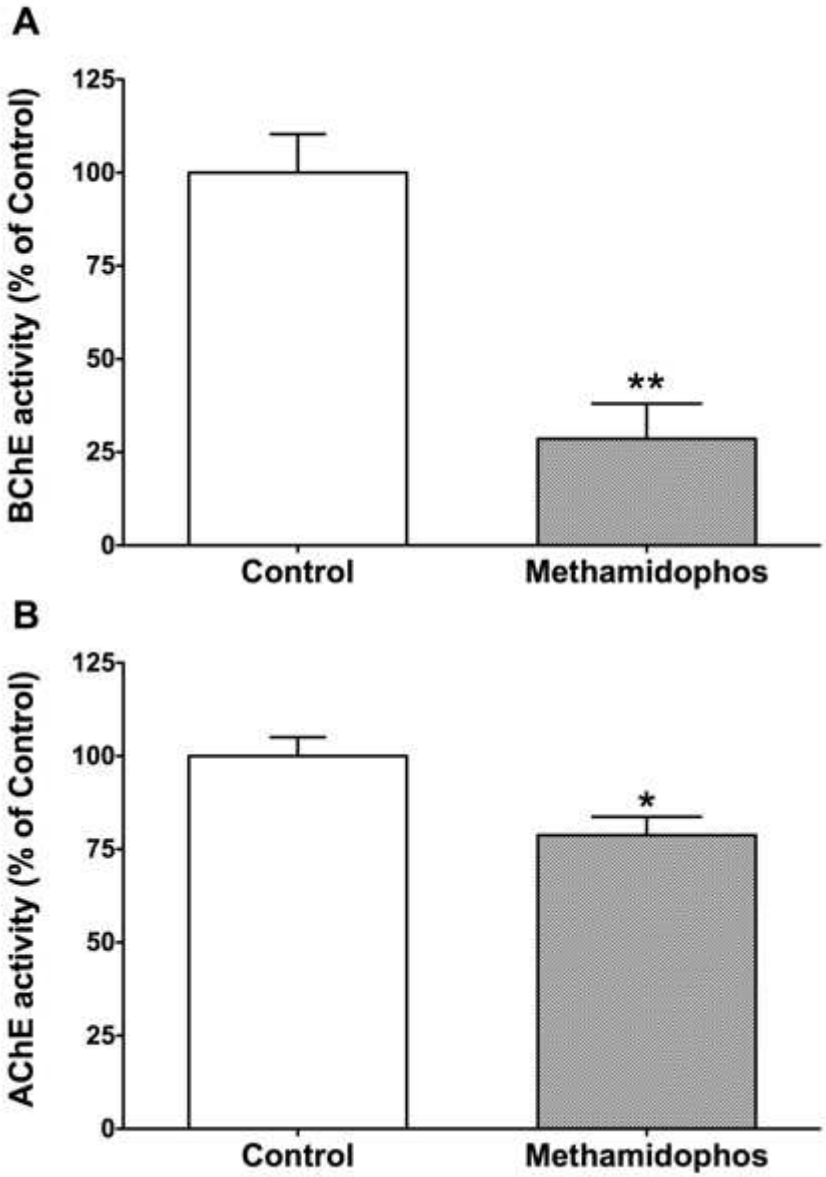
A
BChE activity (% of Control)
125
100
75
50
25
0
**
Control
Methamidophos
B
AChE activity (% of Control)
125
100
75
50
25
0
*
Control
Methamidophos

Figure 4

Table 1

	Behaviour	Treatment Factor	Time Factor	Interaction
Grouped data	Latency to attack	$F_{(1,90)}$=0.04, p>0.05	$F_{(1,90)}$=0.09, p>0.05	$F_{(1,90)}$=0.08, p>0.05
	Number of attacks	$F_{(1,90)}$=1.35, p>0.05	$F_{(1,90)}$=0.37, p>0.05	$F_{(1,90)}$=1.08, p>0.05
	Tail rattling	$F_{(1,90)}$=2.52, p>0.05	$F_{(1,90)}$=1.00, p>0.05	$F_{(1,90)}$=0.200, p>0.05
	Self-grooming	$F_{(1,90)}$=2.81, p>0.05	$F_{(1,90)}$=1.34, p>0.05	$F_{(1,90)}$=0.64, p>0.05
	Rearing	$F_{(1,90)}$=1.50, p>0.05	$F_{(1,90)}$=2.50, p>0.05	$F_{(1,90)}$=0.19, p>0.05
Aggressive animals	Latency to attack	$F_{(1,46)}$=1.20, p>0.05	$F_{(1,46)}$=1.83, p>0.05	$F_{(1,46)}$=0.27, p>0.05
	Number of attacks	$F_{(1,46)}$=0.02, p>0.05	$F_{(1,46)}$=1.40, p>0.05	$F_{(1,46)}$=0.06, p>0.05
	Tail rattling	$F_{(1,46)}$=4.55, p>0.05	$F_{(1,46)}$=2.31, p>0.05	$F_{(1,46)}$=0.41, p>0.05
	Self-grooming	$F_{(1,46)}$=0.12, p>0.05	$F_{(1,46)}$=0.95, p>0.05	$F_{(1,46)}$=1.43, p>0.05
	Rearing	$F_{(1,46)}$=3.14, p>0.05	$F_{(1,46)}$=3.25, p>0.05	$F_{(1,46)}$=0.05, p>0.05
Non-aggressive animals	Latency to attack	$F_{(1,40)}$=2.47, p>0.05	$F_{(1,40)}$=7.24, p<0.05*	$F_{(1,40)}$=2.47, p>0.05
	Number of attacks	$F_{(1,40)}$=4.32, p<0.05*	$F_{(1,40)}$=5.28, p<0.05*	$F_{(1,40)}$=0.08, p<0.05*
	Tail rattling	$F_{(1,40)}$=0.22, p>0.05	$F_{(1,40)}$=1.39, p>0.05	$F_{(1,40)}$=0.08, p>0.05
	Self-grooming	$F_{(1,40)}$=2.80, p>0.05	$F_{(1,40)}$=0.64, p>0.05	$F_{(1,40)}$=0.08, p>0.05
	Rearing	$F_{(1,40)}$=0.001, p>0.05	$F_{(1,40)}$=3.72, p>0.05	$F_{(1,40)}$=0.15, p>0.05

Table 2

Treatment	Crossed Squares	
	Central	Peripheral
CTRL-Sal	21.2 ± 4.8	60.3 ± 5.0
MTD 3.5 mg/Kg	10.5 ± 1.9 #	68.8 ± 7.6

Notes: Data represent the mean ± S.E.M. # p=0.052 from the MTP 3.5mg/Kg compared with saline group.